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A Molecular Dynamic Study of the Basic Fibroblast Growth Factor - Fibroblast Growth Factor Receptor Complex

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The basic fibroblast growth factor is a 155 amino acid residues peptide that plays versatile functions throughout the human life cycle. It binds to its tyrosine kinase-containing FGF receptor, FGFR1, and to heparan sulfate proteoglycans. The presence of ethanol interferes on the bFGF biologic activities resulting in numerous anomalies like the fetal alcohol syndrome. Experimental studies suggest that the interaction sites between bFGF and FGFR1 are the target for ethanol molecules. In order to better understand the interaction between bFGF, FGFR1 and ethanol, molecular dynamics simulations of the complex bFGF-FGFR1-heparin in water and in water-ethanol solutions were carried. Hydrogen bond structures, root mean square deviations and specific radial distribution functions were employed to identify the role of each component on the complex stability. Some residues (SER73, TYR112, THR114, TYR115 and SER117) were identified as extremely important for the perfect linking between the bFGF and FGFR1. There are exactly these residues that are the target of ethanol molecules resulting in the impossibility to occur the docking of bFGF on its receptor.

1 Introduction

The cell growth is a tightly regulated process controlled by highly specific proteins, namely the growth factors[1]. The bFGF, a mammalian fibroblast growth factor, is a sequence of 155 amino acid residues that plays versatile functions throughout the human life cycle. Two types of molecules bind bFGF to mediate its response: (1) tyrosine kinase-containing FGF receptor, FGFR1, and (2) heparan sulfate proteoglycans (heparin and heparan sulfates, HS). FGFR1 is an integral membrane protein composed by an extracellular ligand binding segment consisting of three immunoglobulin-like domains (D1, D2 and D3), a single transmembrane helix and a cytoplasmic portion that contains a largely split tyrosine kinase activity[2]. The ligand binding and the specificity residues of the FGFR1 are located in the D2 and D3 domains and in the linker that connects these domains. The bFGF:FGFR1 complex contains a 2:2 dimeric bFGF-FGFR1-HS assemblage. D2 and D3 are sufficient for the bFGF binding and to determine its specificity[3]. Experimental studies on bFGF biologic activities revealed that the presence of ethanol interferes in its activity apparently because the interaction sites between bFGF and FGFR are the targets of the ethanol molecules[2]. The objectives of the present study are (1) to describe the structure of bFGF-FGFR1 interactions and (2) to contribute to better understand the structural factors that determine the loss of bFGF activity in the presence of ethanol molecules. Molecular dynamics simulations[4] conducted with the Gromos96 force field and the GROMACS 3.2 package[5] will be used. The simulated systems consisted of one dimer bFGF-FGFR1 (initial guess: pdb code 1FQ9) immersed in water or in a mixed water-ethanol medium.The solvent concentrations

were adjusted to match, in the pure solvent regions of the simulation systems, their experimental densities. Na^+ and Cl^- ions were added to preserve the local electroneutrality. After an initial phase, the 10.0 ns long simulations were conducted at 298 K and 1 atm in a 13.5 x 13.5 x 13.5 nm^3 simulation cell, implementing the PME method with a cut-off applied at 1.4 nm.

2 Results and Discussion

The structure of the dimer bFGF2-FGFR1 consists of two bFGF molecules (residues 16-144), the dimerized receptor formed by two D2 domains (residues 149-249) linked to two D3 domains (residues 251-359) and two heparin molecules. The structure of the dimer in aqueous medium undergoes only few changes if compared with the starting X-ray structure (RMSD about 0.25nm) while, in ethanol solution, RMSDs with a mean value of 0.60 nm are observed when the dimer structure undergoes significant changes suggesting that some regions are loosing their structure. The characterization, by molecular dynamics simulations, of the peptide structures was carried out by identifying, in a first step, the intramolecular hydrogen bonds (iHB) and, a second step, the intermolecular hydrogen bonds (HB). The dimeric bFGF-FGFR1 structure is stabilized by 20 HB between the dimer and the solvent molecules, 20 between bFGF and FGFR1, 4 between the FGFR1 units, 32 between heparin and the bFGF's and 22 between heparin and the FGFR1's.

The hydration changes in the bFGF-FGFR1 complex comparing the data in aqueous solution and in ethanolic medium reveal decreases of 20% for bFGF and 9% for FGFR1. All the affected residues contain a hydroxyl group in the side-chain and are located in the neighborhood of other residues displaying a hydrophobic character[6](the bFGF residues solvated by ethanol are always the same in the presence or not of the receptor) suggesting that an energetically favorable situation takes place when the ethanol molecules are hydrogen bonded to hydroxyl groups while their methyl groups are accommodated into a hydrophobic environment. In the case of bFGF, the ethanol molecules form intermolecular hydrogen bonds with TYR33 (β1), SER73 (β5), TYR82 (β6), SER109, TYR112(β9), SER114(β9), TYR115(β9), and SER117(β9) so that two important HB between bFGF and FGFR1 D2 domain are lost: the links between SER109 of bFGF and ASP209 of FGFR1 and between TYR112 (β9) of bFGF and ALA179 located in the FGFR1 D2 domain.

The solvation by the ethanol molecules shows a more active interference of the ethanol molecules with the residues belonging to the bFGF β1, β5, β6, β9, β10, and β12 strands. The most important interference (*i.e.,* the longer residence times) is observed on the β1 (TYR33), β6 (TYR82), β9 (SER109, TYR112, TYR115 and SER117) residues. The consequence is the lost of four bFGF-FGFR1 HB, three between each one of the bFGF's and the D2 domains and one HB between each bFGF and the D3 domains. The losses of local structures are confirmed by the experimental results that also suggest that the interaction between bFGF and FGFR is the target for ethanol in its inhibition process. On the basis of the detailed description of the interactions of bFGF-FGFR1 complex with the ethanol molecules, it can be establish that the residues belonging to the β1, β6, and β9 strands, especially TYR33, TYR82, SER109, and TYR112 are extremely important for the perfect linking between the bFGF and FGFR1.

3 Concluding Remarks

The studies by molecular dynamics of the bFGF-FGFR1 complex structure conclude that the ligand binding and specificity require both D2 and D3 domains of FGFR1, that the heparin molecules are a fundamental binding element between bFGF and the receptor, that the interaction of bFGF with its membrane receptor must be described by a HB complex network where all HB are essential for the perfect bFGF binding to FGFR1 (the perfect binding that is an unavoidable step to bFGF exhibits all its biological activities). The simulation results confirm the experimental data concerning the importance of the TYR-112 and ASN-113 in the bFGF activity but also are able to identify the SER73, TYR112, THR114, TYR115 and SER117 residues as being probably important to understand the activity of the bFGF growth factor.

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